REMARKS/ARGUMENTS

Claims 1-67 are pending in the captioned application. Claims 23-42 and 59-67 are withdrawn from consideration. Claims 1-22 and 43-58 are under examination.

The Examiner stated, "The information disclosure statement filed March 25, 2002 and November 17, 2003 is acknowledged. However, The priority document A22 and A23 was not considered by the Examiner because the documents could not be found in the instant application. Additionally, the document listed as Bl was not considered by the Examiner because and English language translation of the document was not provided by Applicant".

In response, Applicants are enclosing, concurrently herewith, documents A22 and A23, as well as a copy of US. Patent number 5,595,741 which is an English translation of the cited reference listed as document B1. Consideration of these references is respectfully requested.

The Examiner has rejected claims 1-22 and 43-58 under 35 U.S.C. § 112, second paragraph, "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

(a) Claims 1-22 and 43-58 are indefinite because the claims lack a final process step which clearly relates back to the preamble. The claims are drawn to 'a method of detecting a target nucleic acid or protein' or 'a method of detecting a single nucleotide polymorphism', whereas the final step recites 'detecting the fluorophore'. Thus it cannot be clearly determined if the method is directed to detecting a target molecule or polymorphism or detecting specific signal. It is suggested amending the claims to recite a final process step which clearly relates back to the preamble and for consistency of claim language".

In response, Applicants have amended claims 1 and 43 as the Examiner has suggested. Since all claims are ultimately dependent upon claim 1 or 43, all claims now contain this limitation.

In view of the foregoing, Applicants respectfully assert that the Examiner's rejection can not be sustained and should be withdrawn.

The Examiner has also stated, "(b) Claims 1 -22 are confusing in claim 1 because the claim 1 lacks a correlative step which links a first member of a binding pair to a target nucleic acid or target protein. The claim 1 as written only requires that a first member of a binding pair be provided and subsequently contacted with a second member of a binding pair and detected. Thus it cannot be determined how the steps operate to detect a

target nucleic acid or protein in the context of the claim language. Clarification is required".

In response, Applicants respectfully submit that the claims state specifically what the Examiner wants. Specifically, in step (a), it is stated that the target provided comprises "a nucleic acid or protein and a first member of a binding pair". This target is then hybridized to a probe attached to a hydrogel, and a second member of the binding pair, which comprises a fluorophore, is then attached to permit the detection to occur. Nevertheless, in an effort to make the claim clearer, step (a) has been amended to recite "providing a target comprising a target nucleic acid or protein".

In view of the foregoing, Applicants respectfully assert the Examiner's rejection can not be sustained and should be withdrawn.

The Examiner further states "(c) Claims 12, 13 and 50 are indefinite at the recitation of 'capable of' because it cannot clearly be determine whether the limitation after 'capable of' is a property of the 'probe' or the 'reactive site' or whether the limitation is a separate entity. Clarification is required".

In response, Applicants respectfully assert that the claims are clear as written. Specifically, claim 12 recites that the probe comprises a reactive site capable of

undergoing a 2 + 2 photocycloaddition, while claim 13 recites that the hydrogel matrix comprises a reactive site capable of undergoing a 2 + 2 photocycloaddition. Inasmuch as claim 1 recites that the probe is attached to the hydrogel through a 2 + 2 photocycloaddition, it is clear that claim 12 contains the limitation that the probe include the reactive site, while claim 13 contains the limitation that the hydrogel matrix includes the reactive site. Claim 50 also states that the probe comprises a reactive site capable of undergoing a 2 + 2 photocycloaddition, while claim 43 specifically states that the probe is attached to a hydrogel matrix through a 2 + 2 photocycloaddition. Accordingly, Applicants respectfully assert that the claims are proper as written.

In view of the foregoing, Applicants respectfully assert the Examiner's rejections can not be sustained and should be withdrawn.

The Examiner further states, "No claims are allowed. However, the claims are free of the prior art because the prior art does to teach the step of hybridizing a target nucleic acid or protein to a probe attached to a hydrogel matrix through a 2 + 2 photocycloaddition". Applicants gratefully acknowledge this finding, and respectfully assert that above amendments and arguments overcome the rejections under 35 U.S.C. § 112.

In view of the foregoing, Applicants respectfully assert the Examiner's rejections can not be sustained and should be withdrawn. Applicants believe that claims 1-22 and 43-58, as amended, are in allowable form and earnestly solicits their allowance.

Early and favorable action is earnestly solicited.

Respectfully submitted,

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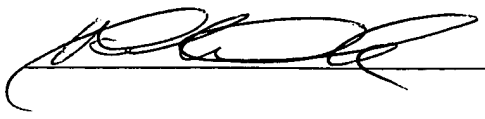
Signature: 

Name: Melissa Leck

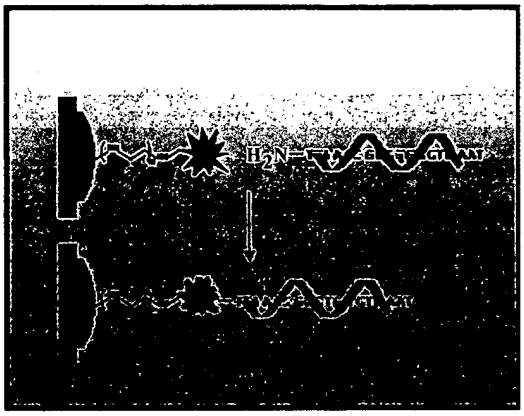
A22

Technical Note

DNA Immobilizer™

No. DNA01/11.98

By: Nana Jacobsen, M.Sc. and Jan Skouv, Ph.D.



The DNA Immobilizer™ is manufactured using Exiqon's patented photochemical method[1] for covalent coupling of ligands to polymer materials. The photocoating technique introduces an ethylene glycol spacer and a stable electrophilic group that reacts with nucleophiles such as free amines. The spacer design and the density of electrophilic groups on this surface are optimised for detection of various types of nucleic acids.

Recommended coupling protocol

Materials:

- DNA Immobilizer™ microplate.
- 100 mM carbonate buffer, pH=9.6 (coupling buffer).
- Amino coupled detection-DNA e.g. NH_2-oligos or
- NH_2-PCR™ fragments.
- SSC (1xSSC is: 150 mM NaCl, and 15mM sodium citrate).

Oligonucleotides with either a 3' or 5' NH_2-group can be obtained from most suppliers of oligonucleotides. NH_2-PCR fragments may be synthesised in a standard PCR reaction including one 5'-NH_2-primer.

Protocol for 96 well plate, 8 well strips, and 16 well strips:

1. Prepare a solution of your amino coupled DNA in 100 mM carbonate buffer, pH=9.6. We recommend that the amount of aminated catching DNA is optimised. Initially, we suggest the following: 0.1 µM of aminated DNA oligos or 1 nM of aminated PCR-fragments.
2. Add the NH_2-DNA solution to the wells of the DNA Immobilizer™ microplate (100 µl/well).
3. Incubate the plate with gentle agitation at room temperature for 2 hours or overnight at +4°C.
4. Aspirate the wells and wash with 3x300 µl 2xSSC, 0.1% (v/v) TWEEN® 20.
5. Your DNA surface is ready for use.

Protocol for 384 well plate:

1. Prepare a solution of your amino coupled DNA in 100 mM carbonate buffer, pH=9.6. We recommend that the amount of aminated catching DNA is optimised. Initially, we suggest the following: 0.1 µM of aminated DNA oligos or 1 nM of aminated PCR-fragments.
2. Add the NH_2-DNA solution to the wells of the DNA Immobilizer™ microplate (50 µl/well).
3. Incubate the plate with gentle agitation at room temperature for 2 hours or overnight at +4°C.
4. Aspirate the wells and wash with 3x300 µl 2xSSC, 0.1% (v/v) TWEEN® 20.
5. Your DNA surface is ready for use

The DNA will be immobilised via the terminal NH_2-group.Detergents like TWEEN® 20 effectively suppress covalent coupling of DNA. Detergents should not be present in the coupling buffer. The use of competing nucleophiles like ethanolamine, lysine, or tris(hydroxymethyl)aminomethane (TRIS) should also be avoided in the coupling buffer.
It is recommended to include small amounts of detergents like TWEEN® 20 (0.05-1% (v/v)) in wash and assay buffers, as this generally improves the signal to noise ratio of the assay. Other DNA concentrations, incubation times, temperatures, buffers or pH-values than those recommended here can successfully be used.

Application example

Detection of a PCR™ amplicon

To illustrate various aspects of the DNA Immobilizer™ performance detection of a PCR amplicon was set up, see Figure 1 and 2.

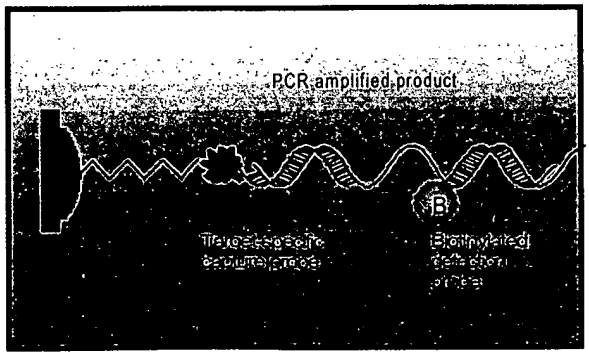


Figure 1: Detection of a PCR™-fragment by a target-specific capture probe covalently linked to the DNA Immobilizer™ microplate and a biotinylated detection probe.

The DNA-fragment to be detected was a 98 bp fragment from pUC19[2,3]. This 98 bp fragment was amplified using 5'-AAC AGC TAT GAC CAT G-3' and 5'-GTA AAA CGA CGG CCA GT-3' as primers, pUC19 as template and a standard PCR™ kit (Boehringer Mannheim cat. no. 1647679). The fragment was amplified following the manufacturer's recommendations and incubating: 2 min at 94°C; 30 cycles (94°C 1 min, 45°C 1 min, 72°C 2 min); and 72°C 3 min. The yield was estimated by agarose gel electrophoresis.

Detection of PCR™ amplicon

1. Using the recommended coupling protocol for the DNA Immobilizer™ 96 well plate, the catching sequence 5'-amine-AAC AGC TAT GAC CAT G-3' was covalently attached to the transparent DNA Immobilizer™ microplate surface.
2. Per well: 10 µL of the PCR™ reaction (approximately 40 ng) was dissolved in 2XSSC, 0.1% (v/v) TWEEN® 20. Boiled 5 min. and then placed on ice.
3. The specific detection probe (5'-biotin-ATG CCT GCA GGT CGA C-3') was added to the PCR™/SSC mix. 0.5 pmol detection probe per µL, final vol. 100 µL. The mix was then added to the wells of the DNA Immobilizer™ microplate and the PCR-fragment was allowed to hybridise to the covalently attached catching sequence for 3 hours at 37°C.
4. The wells were aspirated and washed with 3X300 µl 2XSSC, 0.1% (v/v) TWEEN® 20.
5. A 1 µg/ml solution of streptavidin/HRP (Pierce cat. no. 21126) in PBST (Phosphate Buffered Saline[4] with 0.05% (v/v) TWEEN® 20) was dispensed into the wells (100 µl/well) and the plate incubated for 1 hour.
6. The wells were aspirated and washed with 3X300 µl PBST.
7. A solution of 6 mM ortho-phenylene-diamine (OPD), 4 mM H_2O_2 in 100 mM citric acid buffer, pH=5.0 was added to the wells (100 µl/well) and left for colour development.
8. After approximately 15 minutes the enzyme reaction was stopped with H_2SO_4, 0.5 M (100 µl/well) and the optical density in this colorimetric assay was measured at λ=492 nm with an ELISA-reader.

All incubations were carried out with gentle agitation at either room temperature or at 37°C when indicated.

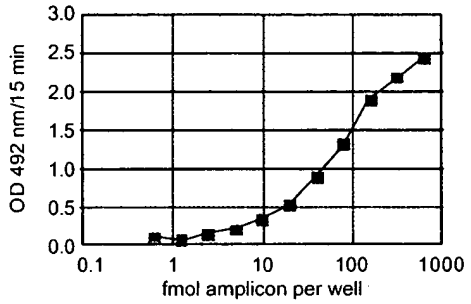


Figure 2: Detection of various amounts of the 98 bp PCR™ fragment from pUC19

References:

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3) pUC 19 accession no.: VB0026.
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Last revision: December 29, 2000

A23

Technical Note

DNA Immobilizer™

No. DNA02/04.99

By: Nana Jacobsen, M.Sc. and Jan Skouv, Ph.D.

Immobilization of Double-stranded PCR-Amplicon to the Surface of the DNA Immobilizer™

With the advent of polymerase chain reaction (PCR®), ligase chain reaction (LCR[1]), and similar techniques, double-stranded (ds) DNA fragments with a well defined DNA-sequence can be prepared. In particular the efficient generations ofdsDNA fragments by PCR have found numerous applications in diverse fields of biomedicine and molecular biology.

Oligos with an amino-group attached to its 5'-end can be purchased from most commercial oligo-suppliers. Including one such oligo-primer in a PCR® (or LCR) reaction result in the synthesis of amino-labelled dsPCR®-fragment. Such fragments can easily be covalently linked to the surface of the DNA Immobilizer™ microplates and strips and used for various applications, e.g.:

Enzymatic activity:
A number of important enzymes, for instance restriction enzymes, kinases, phosphatases, polymerases, methylases etc. act on DNA. The possible relation between enzymatic activity and specific DNA-sequences can conveniently be tested on DNA's covalently linked to the DNA Immobilizer™.

Analysis of DNA binding proteins:
We suggest thatdsDNA's with various recognition sequences are generated and covalently attached to the DNA Immobilizer™ surface. Subsequently various (labelled) protein-fractions may be presented to the immobilised DNA and theDNA:Protein association analysed. One of the favourable characteristics of the DNA Immobilizer™ is its very low capacity for non-specific protein binding in buffers containing even diminutive amounts of TWEEN®20.

Gene discovery:
A number of gene discovery methodologies (e.g. differential display[2] result in a large number of PCR-fragments that have to be screened for the presence of a given consensus sequence. We suggest to attach such PCR-fragments generated with one amino-labelled oligo to the surface of the DNA Immobilizer™ and screen for the presence of a particular DNA sequence as described below.

Recommended coupling protocol

Materials:

- DNA Immobilizer™ microplate
- 100 mM carbonate buffer, pH=9.6.
- Amino coupled PCR amplicon.
- SSC (1×SSC is: 150 mM NaCl, 15 mM sodium citrate, pH 7.0).
- PBST, PBST is Phosphate Buffered Saline[6] (PBS) with 0.05% (v/v) TWEEN®20.

Coupling Protocol for 96 well plate:

1. Prepare a solution of your amino coupled DNA in 100 mM carbonate buffer, pH=9.6. It is recommended that the amount of aminated DNA is optimised, however for initial experiments we suggest 1 nM of aminated DNA PCR®-fragments.
2. Add the NH_2-DNA solution to the wells of the DNA Immobilizer™ microplate (100 µl/well).
3. Incubate the plate with gentle agitation at room temperature for 2 hours or over night at +4°C.
4. Aspirate the wells and wash with 3×300 µl 2×SSC, 0.1% (v/v) TWEEN®20
5. Your DNA surface is ready for use.

Detergents like TWEEN®20 effectively suppress covalent coupling of DNA and should consequently not be present in the coupling buffer. The use of competing nucleophiles like ethanolamine, lysine, or tris(hydroxy-methyl)aminomethane (TRIS) should also be avoided in the coupling buffer.
It is recommended to include small amounts of detergents like TWEEN®20 (0.05-1% (v/v)) in wash and assay buffers, as this generally improves the signal to noise ratio of the assay.
Other DNA concentrations, incubation times, temperatures, buffers or pH-values than those recommended here can successfully be used.

Application example:

Immobilisation and Detection of an Amino-labelled PCR Amplicon on the surface of DNA Immobilizer™.

To illustrate various aspects of the performance of the DNA Immobilizer™ detection of an amino-PCR amplicon was set up.

The DNA-fragment to be detected was a 630 bp fragment from human N-*ras*[3,4]. This 630 bp fragment was amplified using 5'-NH_2-C6-spacer-CCA GCT CTC AGT AGT TTA GTA CA-3' (position 1427-1449) and 5'-AAG TCA CAG ACG TAT CTC AGA C-3' (position 2035-2056) as primers, humanN*ras* as template and a standard PCR kit (Boehringer Mannheim cat. no. 1647679). All oligos were purified by HPLC. The fragment was amplified following the manufacturer's recommendations and incubating 3 min at 95°C; 30 cycles (55°C 2 min, 72°C 3 min, 95°C 1 min); 55°C 2 min and 72°C 3 min. The yield was

estimated on a standard 1% agarose gel stained with ethidium bromide.

Detection of amino-PCR amplicon

1. Using the recommended coupling protocol described above for a 96 well plate (384 well plate coupling protocol please see Technical Note DNA01), the NH_2-N*ras* amplicon was covalently attached to the DNA Immobilizer™ microplate. 10 µl of the PCR® reaction (approximately 120 ng) was diluted in two fold dilutions in 100 mM carbonate buffer, pH=9.6 and 100 µl was dispensed per well. Incubation for two hours was allowed.
2. After coupling the amplicon was denatured by 200 µl 0.4 M NaOH 0.25% (v/v) TWEEN®20 for 5 minutes and washed with 3×300 µl 2×SSC, 0.1% (v/v) TWEEN®20. The N*ras* DNA was detected by hybridisation with the specific detection probe (5'-TGT GTT TGT GCT GTG GAAGAA CCC-biotin-3', position 1549-1572. The probe was diluted in 2 x SSC, 0.1% (v/v) TWEEN®20 final concentration. 100 µl 0.5 µM probe was added per well of the DNA Immobilizer™ microplate. The detection probe was allowed to hybridise to the covalently attached sequence for 2 hours at 37°C.
3. The wells were aspirated and washed with 3×300 µl 2×SSC, 0.1% (v/v) TWEEN®20
4. A solution of streptavidin/HRP (Pierce cat. no. 21126) in PBST (1µg/ml) is dispensed into the wells (100µl/well) and the plate is incubated for 1 hour. The wells were aspirated and washed with 3×300 µl PBST.
5. A solution of ortho-phenylene-diamine (OPD tablets, KemEnTec DK), 6 mM and H_2O_2, 4 mM in 100 mM citric acid buffer, pH=5.0 was added to the wells (100 µl/well) and left for colour development.

After approximately 5 minutes, the enzyme reaction was stopped with H_2SO_4, 0.5 M (100 µl/well) and the optical density was measured at λ=492 nm with an ELISA-reader.

All incubations are carried out with gentle agitation at either room temperature or at 37°C when indicated.

Results

The result of a typical experiment is shown in Figure 1. The experiment indicates that at high concentrations of the amino-amplicon the hybridisation signal decreases to the background level. This effect appears somewhat similar to the 'high-dose hook effect' described for various immuno-assays[7-9], and emphasises that an optimisation of amino-amplicon concentrations should be made.

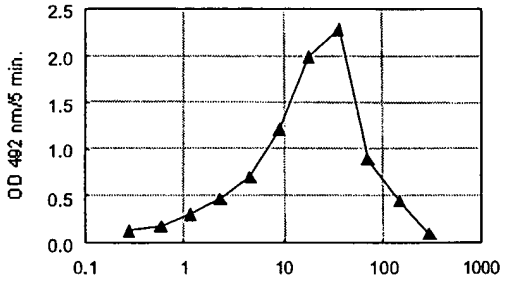

Figure 1: Detection of various amounts of the 630 bp PCR fragments from Nras.

References

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